Screening Fresh Forages for Protein Degradation and Nutritive Value

Garry C. Waghorn
AgResearch, New Zealand

J. L. Burke
Massey University, New Zealand

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G.C. Waghorn[1] and J.L. Burke[2]

[1]AgResearch Grasslands, Private Bag 11008, Palmerston North, New Zealand. waghorng@Agresearch.cri.nz

[2]Massey University, Private Bag 11000, Palmerston North, New Zealand

Abstract

A method has been developed to prepare fresh forages for *in sacco* and *in vitro* incubation by freezing and mincing to achieve a particle size distribution of dry matter (DM) similar to *in vivo* conditions. The method is described and data presented to indicate losses of nitrogen (N) during *in sacco* digestion and net yield of ammonia from proteolysis *in vitro* for 22 fresh and conserved forages. Grasses, legumes and herbs were evaluated, with fractional degradation rates of forages ranging from 0.07h^{-1} with tall fescue (*Festuca arundinacea*) to 0.24 h^{-1} with chicory (*Cichorium intybus*). Degradation of protein to ammonia *in vitro* over 24 h was highest with white clover (*Trifolium repens*) and about 20% of the nitrogen (N) released during degradation was incorporated into microbial N. These data will assist formulation of forage based total mixed rations for high producing ruminants.

Keywords: Forage, *in sacco*, *in vitro*, digestion, feed quality

Introduction

The importance of *in vitro* and *in sacco* incubations of ruminant feedstuffs to estimate nutritive value, and digestion characteristics, establish kinetic parameters for rates of degradation and to determine products of fermentation is unquestionable. More controversial is the form, or preparation of feedstuffs used for incubations. In most instances, material is dried and ground through a 1mm sieve prior to incubation. This may be reasonable for dry feeds, but it is inappropriate for fresh material.

McNabb et al. (1996) Compared freeze-dried (FD) with fresh minced (FM) forage and showed preparation had a major effect on fermentation characteristics. *In sacco* incubations resulted in a greater loss of N from FM than FD material at all times over 24 h, with initial N losses of 0.47 and 0.15, respectively. The extent to which fresh leaf is disrupted is also important, with *in vitro* gas production about 20% higher in freshly chewed than whole leaf of legumes, and homogenising resulted in 30% more gas production than from chewed material (Fay et al 1980).

Information presented here summarises some aspects of method development for mincing and incubating forages and provides comparative data between 22 contrasting forages (including some silage) for rates of protein degradation. Our objective was to define kinetic characteristics of forage degradation, including products arising from digestion, to better meet nutrient requirements of high producing forage fed to ruminants. Data presented here summarise N aspects of digestion.

Material and Methods

The particle size distribution (by wet sieving) in minced feed preparations was based on rumen contents and boli swallowed after chewing during eating by sheep fed either perennial ryegrass (*Lolium perenne*) or white clover. Mincers varied in performance, especially the

configuration of sieve plates and hole size, and data are presented here for a Krefft compact (Germany) with 12 mm holes, which achieved a particle size distribution similar to that of sheep digesta.

Preliminary measurements of bacterial growth during *in vitro* incubation were made with finely chopped white clover, perennial ryegrass and *Lotus corniculatus* using a bacterial DNA probe and serial dilutions of rumen bacteria to quantify numbers and nitrogen content *in vitro*. Bacterial N was measured after 0, 2, 8 and 24 h (4 samples for each time point) to determine the extent to which plant N was incorporated into bacteria *vs.* ammonia pools.

About 600g fresh forage was required for *in sacco* and *in vitro* incubations. Material was frozen immediately following collection, chopped into 20-30mm lengths and minced in a cold (-20^{o}C) mincer to prevent thawing during preparation or weighing into bags (Ankom, USA) or bottles for incubation. Bag size was 100 x 100mm and contained about 30g wet material (5-8g DM) which ensured sufficient residues were available from duplicate bags after 24h incubation for NIRS (near infra red spectroscopy) analysis, without over filling bags. *In vitro* incubations were carried out in 50ml Schott® bottles fitted with gas release valves with about 2.5 g wet material (0.4-0.6g DM), 12ml McDougals buffer, 3ml rumen liquor and 0.5ml cysteine sulphate reducing agent (Burke et al., 2000). Tests showed inclusion of the reducing agent reduced variation by half between replicates in ammonia production.

Twenty-two forages (16 fresh, 6 conserved) have been prepared by mincing. The forages (Table 1) comprised grasses, legumes and herbs and were subject to 72h *in sacco* and 24h *in vitro* incubation with routine sampling as described by Burke et al (2000). Nitrogen disappearance was analysed by fitting bag residue data to the model: $P = A + B\ (1-e^{-k\ (t-L)})$ where P is potentially degradable N, A is the soluble N portion, B is insoluble potentially

degradable N, K is the fractional disappearance rate (h^{-1}), t = incubation time (h) and L = lag time (h). Ammonia yield (in vitro) was determined at 2 h intervals to 12 h and at 24 h.

Results

The particle size distribution for chewed forage and rumen contents of sheep fed white clover, ryegrass and the fresh minced forage covers range of values because of differences between chewed material and rumen contents. Particle sizes have been defined in terms of sieve aperture size, residual DM (smaller than 0.25 mm, but not soluble) and soluble DM: 4 mm, 1-4 mm, and 0.25-1 mm, residual, soluble. Respective ranges for distribution of DM from chewed boli and rumen contents from sheep fed ryegrass were 14-42%, 3-11%, 7-15%, 6-27% and 33-41%, compared to 22, 18, 14, 10 and 35% of DM from minced ryegrass. When sheep were fed white clover the DM distribution was 11-33, 4-18, 9-14, 5-32 and 35-42% soluble, compared to 8, 33, 21, 4, and 34% of DM from minced clover. Inclusion of rumen contents in the definition of forage particle size distribution is important because it replicates reality, and incorporates effects of rumination, which will compensate for variations in particle size due to eating rate.

There were no differences between white clover, ryegrass and *Lotus corniculatus* in the percentage of plant N incorporated into bacteria after 2 h (2.8%) and 8 h (6.6%), but at 24 h 10.7, 6.3 and 8.8% of plant N were incorporated into bacterial N for the respective forage species. The N associated with bacterial growth corresponds with the net yield of ammonia from *in vitro* incubations of the three forages at 2 and 8 h (Figure 1). After 12 h of incubation the net yield of ammonia increased only slightly for most forages. Exceptions were white clover, red clover (*Trifolium pratense*) and alfalfa, which had in excess of 45% plant N released to ammonia N after 24h of incubation.

The data in Table 1, which include dry matter N content, and N losses to digestion, complement losses to ammonia in Figure 1. The rate constants (k) are based on a model, which includes a lag component and range from as little as 0.03 h^{-1} with maize silage to 0.24 h^{-1} with chicory, which suggest a large diversity between forages in rates of proteolysis *in vivo*.

Discussion

The principal findings outlined here include an appropriate preparation of fresh forages for in vitro and *in sacco* incubation, an indication of N entrapment in microbial protein and development of kinetic parameters to define differences in proteolysis between forages *in vivo*. The importance of this technique, relative to drying and grinding should not be under estimated. For example McNabb et al. (1996) showed microbial N associated with residual DM from *in sacco* incubations was always greater with FD than FM preparations, yet N losses during digestion were always greater with FM than FD preparations. In contrast, excessive breakdown of plant structure by homogenising is equally detrimental to meaningful kinetics, for example by removing inhibitory effects of condensed tannins during digestion (Fay et al., 1980). Fresh minced preparations have been used previously to screen 100 cultivars of white clover and identify those having rapid and slow rates of protein degradation (Waghorn and Caradus, 1994). The kinetic and proteolytic data obtained from the fresh and conserved forages reported here will be used in conjunction with DM, fibre and volatile fatty acid data to formulate balanced forage based diets best able to meet the needs of high producing ruminants.

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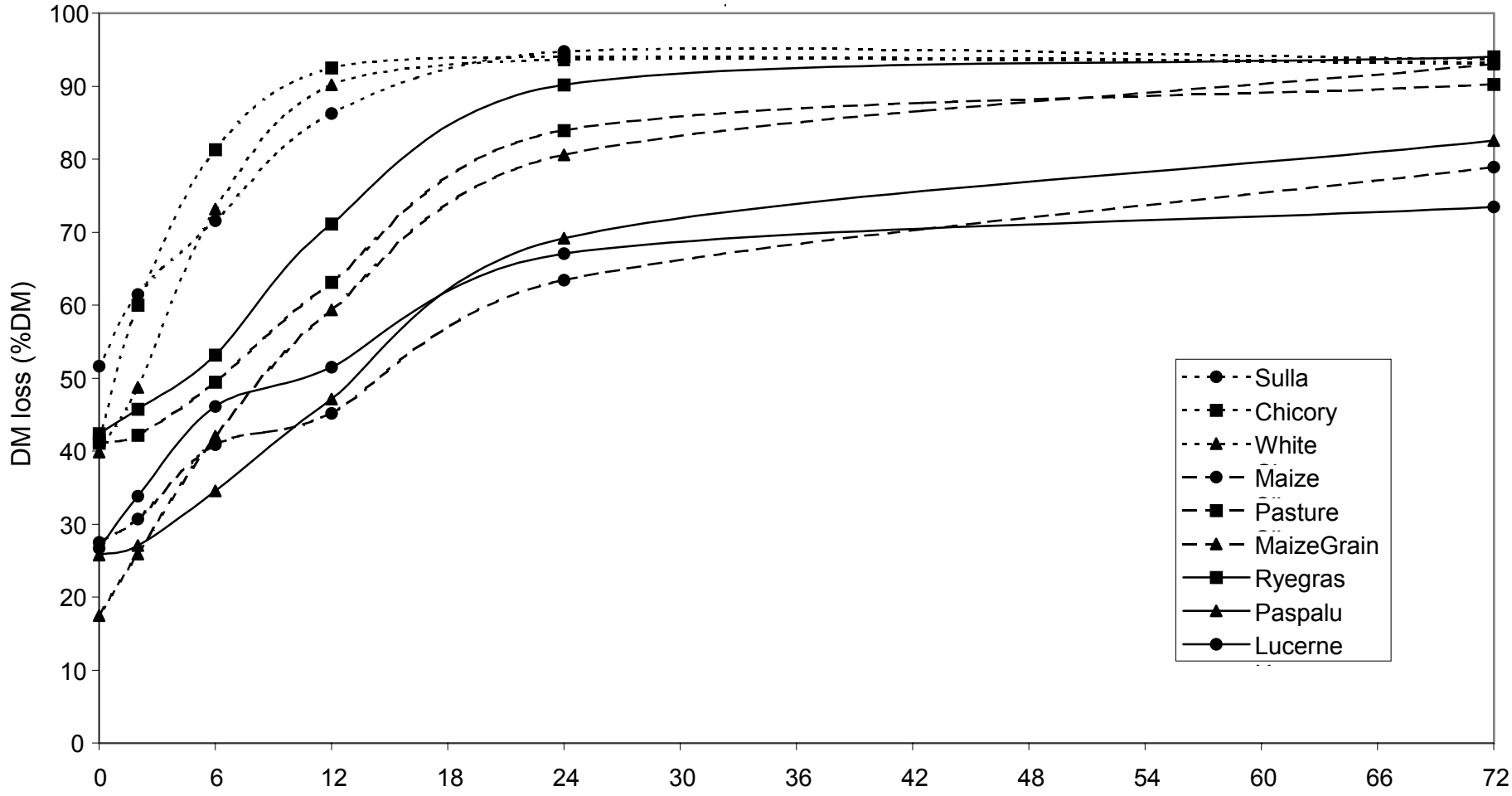
Table 1 - Crude protein (CP) concentration in forages and their nitrogen (N) degradation kinetics *in sacco* (% of total N) as defined by soluble N (A), degradable insoluble N (B), potential N degradability (P), fractional N degradation rate (k, h^{-1}), lag time (L; h) and effective N degradability (E, which assumes a passage rate of 0.06 h^{-1}).

Forage	CP	A	B	P	k	L	E
Fresh	% of DM						
Lolium perenne (Perennial ryegrass)	15.5	52.0	43.6	95.7	0.151	4.54	80.3
Dactylis glomerata (Cocksfoot)	23.7	55.7	40.0	95.7	0.170	5.10	82.2
Festuca arundinacea (Tall fescue)	16.4	52.3	44.5	96.8	0.067	2.40	74.0
Holcus lanatus (Yorkshire fog)	23.7	54.4	44.2	98.5	0.086	0.79	79.8
Bromus willdenowii (Prairie grass)	19.9	52.2	43.3	95.5	0.170	0.79	83.8
Lolium multiflorum (Grasslands Tama)	21.3	56.1	42.3	98.4	0.127	4.00	82.2
Pennisetum clandestinum (Kikuyu)	16.4	55.9	33.9	89.9	0.130	13.52	65.8
Paspalum dilatatum (Paspalum)	13.5	29.5	50.0	79.5	0.096	13.66	40.7
Trifolium repens (White clover)	26.9	38.2	58.4	96.6	0.186	1.23	81.5
Lotus corniculatus (Birdsfoot trefoil)	22.2	50.2	45.4	95.5	0.146	0.79	81.8
Lotus pedunculatus (Lotus major)	21.5	33.0	58.0	91.0	0.110	4.70	66.0
Medicago sativa (Lucerne)	29.9	52.0	41.5	93.5	0.153	0	81.8
Trifolium pratense (Red Clover)	27.4	30.3	65.1	95.1	0.073	0.08	71.6
Hedysarum coronarium (Sulla)	23.0	49.6	46.3	95.9	0.117	0.04	80.2
Cichorium intybus (Chicory)	19.3	28.5	66.5	95.0	0.245	0.49	81.6
Plantago lacelata (Plantain)	24.7	32.3	56.5	88.8	0.279	1.29	78.1
Conserved							
Lucerne silage	23.3	52.3	40.1	92.4	0.139	4.90	77.2
Maize silage	7.6	17.8	62.7	80.5	0.032	4.00	35.8
Oat silage	17.8	72.8	22.4	95.2	0.073	0.07	85.1
Pasture silage	17.2	72.0	23.0	95.0	0.100	5.40	84.2
Sulla silage	21.2	54.2	40.8	94.9	0.066	1.57	74.5
Lucerne hay	24.2	37.5	48.0	85.5	0.101	2.77	65.4


DM loss (%DM)
100
90
80
70
60
50
40
30
20
10
0
0
6
12
18
24
30
36
42
48
54
60
66
72
Sulla
Chicory
White
Maize
Pasture
MaizeGrain
Ryegras
Paspalu
Lucerne

Incubation time (hours)

FIGURE 1. Dry matter degradation curves derived from in sacco incubations for contrasting forages

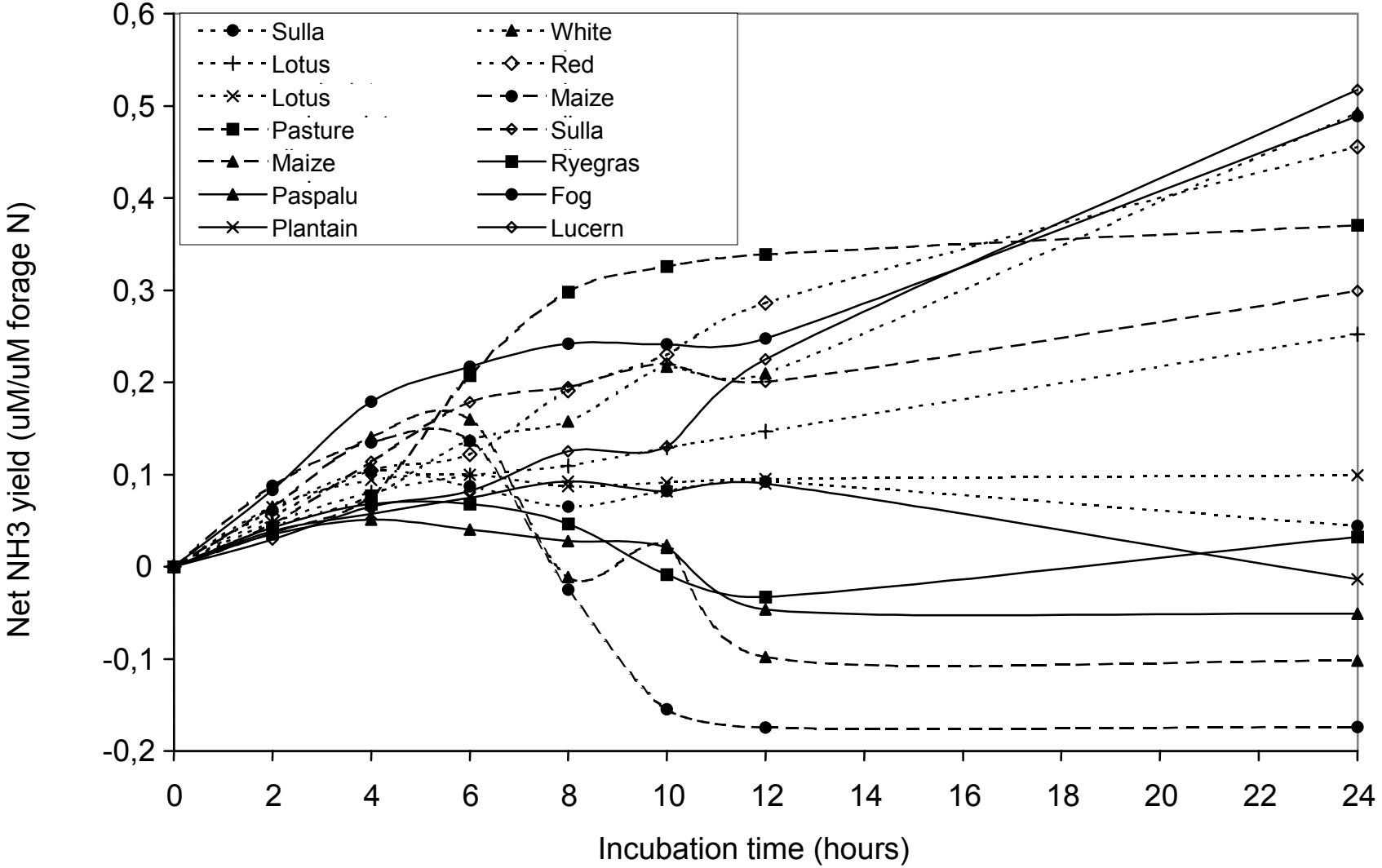


FIGURE 2

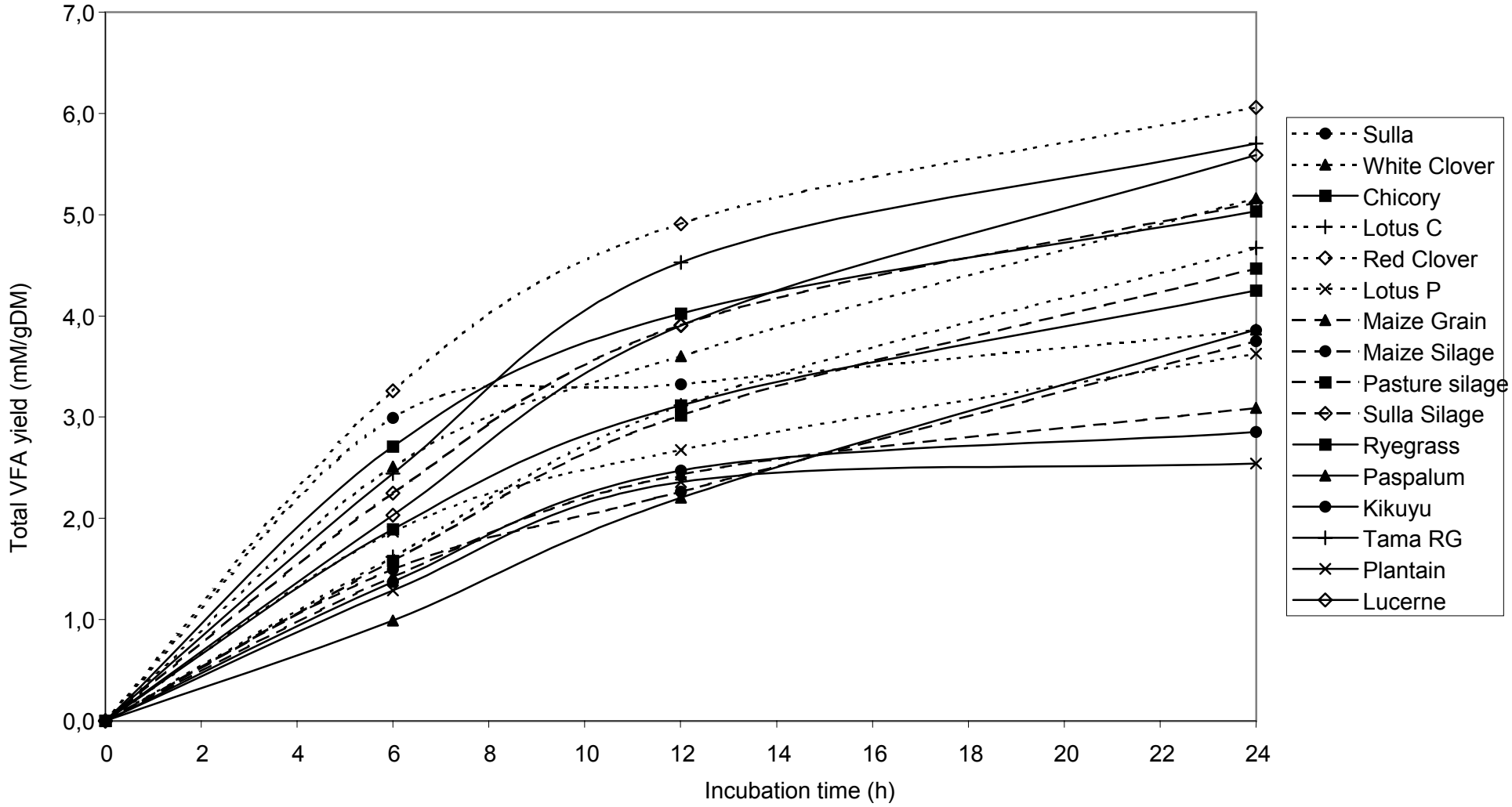


FIGURE 3. Volatiile fatty acid (VFA) yields for contrasting forages